

Serial No. 10/648,152
Docket No. CL2123 US NA

Amendments to the Claims

This listing of claims will replace all prior versions, and listings, of claims in the application:

Listing of Claims:

Claim 1 (currently amended): An isolated nucleic acid molecule ~~encoding an $\alpha(1,6)$ -linked glucose oligosaccharide hydrolyzing polypeptide~~ selected from the group consisting of:

- (a) an isolated nucleic acid molecule encoding the amino acid sequence SEQ ID NOs:2, 4, or 6, wherein said amino acid sequence has $\alpha(1,6)$ -linked glucose oligosaccharide hydrolyzing activity;
- (b) a nucleic acid molecule that hybridizes with (a) under the following hybridization conditions: 0.1X SSC, 0.1% SDS, 65°C and washed with 2X SSC, 0.1% SDS followed by 0.1X SSC, 0.1% SDS; and
- (c) a nucleic acid molecule that is fully complementary to (a) or (b).

Claim 2 (original): The isolated nucleic acid molecule of Claim 1 selected from the group of nucleic acid molecules consisting of SEQ ID NOs:1, 3, and 5.

Claim 3 (withdrawn): A polypeptide encoded by the isolated nucleic acid molecule of Claim 1.

Claim 4 (withdrawn): The polypeptide of Claim 3 selected from the group consisting of SEQ ID NOs:2, 4, and 6.

Claim 5 (currently amended): An isolated nucleic acid molecule encoding an $\alpha(1,6)$ -linked glucose oligosaccharide hydrolyzing polypeptide selected from the group consisting of:

- (a) an isolated nucleic acid molecule encoding a chimeric protein comprised of a signal peptide operably linked to an $\alpha(1,6)$ -linked

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- glucose oligosaccharide hydrolyzing polypeptide having the amino acid sequence set forth in SEQ ID NOs:2, 4, 6, 17, or 31;
- (b) a nucleic acid molecule that hybridizes with (a) under the following hybridization conditions: 0.1X SSC, 0.1% SDS, 65 °C and washed with 2X SSC, 0.1% SDS followed by 0.1X SSC, 0.1% SDS; and
 - (c) a nucleic acid molecule that is fully complementary to (a) or (b).

Claim 6 (withdrawn): The isolated nucleic acid molecule of Claim 5, wherein the signal peptide is SEQ ID NO:24 or SEQ ID NO:25.

Claim 7 (canceled)

Claim 8 (currently amended): The isolated nucleic acid molecule of Claim 5, wherein the signal peptide is SEQ ID NO:24 or SEQ ID NO:25, ~~and wherein the α (1,6)-linked glucose oligosaccharide hydrolyzing polypeptide is SEQ ID NOs:2, 4, 6, 17, or 31.~~

Claim 9 (withdrawn): The isolated nucleic acid molecule of Claim 5, wherein the signal peptide is encoded by the signal sequence as set forth in SEQ ID NO:26 or SEQ ID NO:27.

Claim 10 (original): The isolated nucleic acid molecule of Claim 5 encoding the α (1,6)-linked glucose oligosaccharide hydrolyzing polypeptide, the isolated nucleic acid molecule having the sequence as set forth in SEQ ID NO:1, SEQ ID NO:5, SEQ ID NO:16, or SEQ ID NO:30.

Claim 11 (withdrawn): The isolated nucleic acid molecule of Claim 5 selected from the group consisting of SEQ ID NO:3, SEQ ID NO:28, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, or SEQ ID NO:42.

Claim 12 (withdrawn): The polypeptide encoded by the nucleic acid molecule of Claim 5.

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Claim 13 (withdrawn): The polypeptide encoded by the isolated nucleic acid molecule of Claim 9, Claim 10, or Claim 11.

Claim 14 (withdrawn): The polypeptide of Claim 13 selected from the group consisting of SEQ ID NO:4, SEQ ID NO:29, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:41, and SEQ ID NO:43.

Claims 15-22 (canceled)

Claim 23 (withdrawn): A method for the production of a target molecule in a recombinant host cell comprising:

- (a) contacting a transformed host cell comprising:
 - (i) an isolated nucleic acid molecule encoding a chimeric protein comprised of a signal peptide operably linked to an $\alpha(1,6)$ -linked glucose oligosaccharide hydrolyzing polypeptide;
 - (ii) a nucleic acid molecule that hybridizes with (i) under the following hybridization conditions: 0.1X SSC, 0.1% SDS, 65°C and washed with 2X SSC, 0.1% SDS followed by 0.1X SSC, 0.1% SDS; or
 - (iii) a nucleic acid molecule that is complementary to (i) or (ii); and
 - (iv) at least one chimeric gene for converting monosaccharides to the target molecule,

In the presence of limit dextrin under suitable conditions whereby the target molecule is produced; and

- (b) optionally recovering the target molecule produced in (a).

Claim 24 (withdrawn): A method for the production of glycerol in a recombinant host cell comprising:

- (a) contacting a transformed host cell comprising:

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- (i) an isolated nucleic acid molecule encoding a chimeric protein comprised of a signal peptide operably linked to an $\alpha(1,6)$ -linked glucose oligosaccharide hydrolyzing polypeptide;
- (ii) a nucleic acid molecule that hybridizes with (i) under the following hybridization conditions: 0.1X SSC, 0.1% SDS, 65°C and washed with 2X SSC, 0.1% SDS followed by 0.1X SSC, 0.1% SDS; or
- (iii) a nucleic acid molecule that is complementary to (i) or (ii); and
- (iv) at least one chimeric gene for converting monosaccharides to glycerol,

in the presence of limit dextrin under suitable conditions whereby glycerol is produced; and

- (b) optionally recovering the glycerol produced in (a).

Claim 25 (withdrawn): A method for the production of 1,3-propanediol in a recombinant host cell comprising:

- (a) contacting a transformed host cell comprising:
 - (i) an isolated nucleic acid molecule encoding a chimeric protein comprised of a signal peptide operably linked to an $\alpha(1,6)$ -linked glucose oligosaccharide hydrolyzing polypeptide;
 - (ii) a nucleic acid molecule that hybridizes with (i) under the following hybridization conditions: 0.1X SSC, 0.1% SES, 65°C and washed with 2X SSC, 0.1% SDS followed by 0.1X SSC, 0.1% SDS; or
 - (iii) a nucleic acid molecule that is complementary to (i) or (ii),
 - (iv) at least one chimeric gene for converting monosaccharides to 1,3-propanediol,

in the presence of limit dextrin under suitable conditions whereby 1,3-propanediol is produced; and

- (b) optionally recovering the 1,3-propanediol produced in (a).

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Claim 26 (withdrawn): A method for the production of cell mass in a recombinant host cell comprising:

- (a) contacting a transformed host cell comprising:
 - (i) an isolated nucleic acid molecule encoding a chimeric protein comprised of a signal peptide linked to an $\alpha(1,6)$ -linked glucose oligosaccharide hydrolyzing polypeptide;
 - (ii) a nucleic acid molecule that hybridizes with (i) under the following hybridization conditions: 0.1X SSC, 0.1% SDS, 65°C and washed with 2X SSC, 0.1% SDS followed by 0.1X SSC, 0.1% SDS; and
 - (iii) a nucleic acid molecule that is complementary to (i) or (ii). under suitable conditions in the presence of limit dextrin;
- (b) optionally recovering the cell mass produced in (a).

Claim 27 (withdrawn): The method of Claim 23, Claim 24, Claim 25 or Claim 26 wherein the signal peptide comprises SEQ ID NO:24 or SEQ ID NO:25.

Claim 28 (withdrawn): A method for the production of a target molecule in a recombinant host cell comprising:

- (a) contacting a transformed host cell comprising:
 - (i) an isolated nucleic acid molecule encoding the amino acid sequence selected from the group consisting of SEQ ID NOs:2, 6, 17 and 31;
 - (ii) a nucleic acid molecule that hybridizes with (i) under the following hybridization conditions: 0.1X SSC, 0.1% SDS, 65°C and washed with 2X SSC, 0.1% SDS followed by 0.1X SSC, 0.1% SDS; or
 - (iii) a nucleic acid molecule that is complementary to (i) or (ii); and
 - (iv) at least one chimeric gene for converting monosaccharides to the target molecule,
- in the presence of limit dextrin under suitable conditions whereby the target molecule is produced; and

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- (b) optionally recovering the target molecule produced in (a).

Claim 29 (withdrawn): A method for the production of 1,3-propanediol in a recombinant host cell comprising:

- (a) contacting a transformed host cell comprising:
- (i) an isolated nucleic acid molecule encoding the amino acid sequence selected from the group consisting of SEQ ID NOs:2, 6, 17 and 31;
 - (ii) a nucleic acid molecule that hybridizes with (i) under the following hybridization conditions: 0.1X SSC, 0.1% SDS, 65°C and washed with 2X SSC, 0.1% SDS followed by 0.1X SSC, 0.1% SDS; or
 - (iii) a nucleic acid molecule that is complementary to (i) or (ii); and
 - (iv) at least one chimeric gene for converting monosaccharides to 1,3-propanediol;
- in the presence of limit dextrin under suitable conditions whereby 1,3-propanediol is produced; and
- (b) optionally recovering the 1,3-propanediol produced in (a).

Claim 30 (withdrawn): A method for the production of glycerol in a recombinant host cell comprising:

- (a) contacting a transformed host cell comprising:
- (i) an isolated nucleic acid molecule encoding the amino acid sequence selected from the group consisting of SEQ NOs:2, 6, 17 and 31;
 - (ii) a nucleic acid molecule that hybridizes with (i) under the following hybridization conditions: 0.1X SSC, 0.1% SDS, 65°C and washed with 2X SSC, 0.1% SDS followed by 0.1X SSC, 0.1% SDS; or
 - (iii) a nucleic acid molecule that is complementary to (i) or (ii); and
 - (iv) at least one chimeric gene for converting monosaccharides to glycerol;

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- in the presence of limit dextrin under suitable conditions whereby
glycerol is produced; and
(b) optionally recovering the glycerol produced in (a).

Claim 31 (withdrawn): A method for the production of cell mass in a recombinant
host cell comprising:

- (a) contacting a transformed host cell comprising:
(i) an isolated nucleic acid molecule encoding the amino acid
sequence selected from the group consisting of SEQ ID NOs:2,
6, 17 and 31;
(ii) a nucleic acid molecule that hybridizes with (i) under the
following hybridization conditions: 0.1X SSC, 0.1% SDS, 65°C
and washed with 2X SSC, 0.1% SDS followed by 0.1X SSC,
0.1% SDS; or
(iii) a nucleic acid molecule that is complementary to (i) or (ii),
in the presence of limit dextrin under suitable conditions whereby cell
mass is produced; and
(b) optionally recovering the cell mass produced in (a).

Claim 32 (withdrawn): The method of Claim 28, Claim 29, Claim 30 or Claim 31
wherein the signal peptide is SEQ ID NO:24 or SEQ ID NO:25.

Claim 33 (withdrawn): A method for degrading limit dextrin comprising:

- (a) contacting a transformed host cell comprising:
(i) a nucleic acid molecule encoding the enzymes selected from the
group consisting of SEQ ID NOs:2, 6, 17 and 31;
(ii) a nucleic acid molecule that hybridizes with (i) under the
following hybridization conditions: 0.1X SSC, 0.1 % SDS, 65°C
and washed with 2X SSC, 0.1 % SDS followed by 0.1X SSC,
0.1 % SDS; or
(iii) a nucleic acid molecule that is complementary to (i) or (ii),

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with an effective amount of limit dextrin substrate under suitable growth conditions; and

(b) optionally recovering the product of step (a).

Claim 34 (withdrawn): A polypeptide having an amino acid sequence that has at least 69% identity based on the BLASTP method of alignment when compared to a polypeptide having the sequence as set forth in SEQ ID NO:17, the polypeptide having $\alpha(1,6)$ -linked glucose oligosaccharide hydrolyzing activity.